Amendments to the Specification

Please amend the specification, without prejudice, as follows:

Please replace the paragraph at page 7, lines 22-31, with the following replacement paragraph:

FIG. 3 (comprising FIGS. 3A and 3B): Plasmid map corresponding to pCO14K with a PCR amplification product encoding wild-type PMC21 NhhA operably linked to the *porA* promoter. (Not drawn to scale) FIG. 3A: Solid arrows indicate the arrangement of the *porA* and *kanR* genes in pCO14K. Oligonucleotide primers HOMP5' and HOMP3'AN used to amplify the *nhhA* gene of strain PMC21 are shown. The *nhhA* gene is shown by dotted arrow, the *porA* promoter by a black box, and *Eag*I and *Nco*I restriction sites used to replace *porA* with *nhhA* in as described in Example 2 are shown. FIG. 3B Arrangement of genes in pIP52(PMC21), as described in Example 2. The *Bgl*II site used to construct a mutant as described in Example 4 is shown.

Please replace the paragraph at page 8, lines 1-12, with the following replacement paragraph:

FIG. 4 (comprising FIGS. 4A – 4C): Schematic representation of Splice Overlap Extension PCR strategy for deletion of specific regions of NhhA polypeptides. A schematic of the wild-type *nhhA* gene is shown at the top of Figures 4A-C, and the recombinant *nhhA* is shown at the bottom of these figures, with variable regions shown as black and constant regions by unfilled boxes. Arrows indicate approximate location of oligonucleotide primers. Vertical hatched lines indicate amplification products. Where oligonucleotide sequence is from discontinuous regions of an *nhhA* nucleic acid, this is shown by a dotted line between such discontinuous regions. Approximate scale indicated. Double vertical lines indicate that only a portion of the C5 region is shown. [[A]]FIG. 4A: shows the strategy as described in Example 6. [[B]] FIG. 4B: shows the strategy as described in Example 7. [[C]] FIG. 4C: shows the strategy as described in Example 8.

Please replace the paragraph at page 8, lines 13-15, with the following replacement paragraph:

FIG. 5 (comprising FIGS. 5A and 5B): [[(A)]]FIG. 5A Amino acid sequence of PMC 21 NhhA deletion mutant polypeptide (SEQ ID NO:23) produced in Example 4; and [[(B)]] FIG. 5B encoding nucleotide sequence (SEQ ID NO:28).

Please replace the paragraph at page 8, lines 16-18, with the following replacement paragraph:

FIG. 6 (comprising FIGS. 6A and 6B): [[(A)]]FIG. 6A Amino acid sequence of H41 NhhA deletion mutant polypeptide (SEQ ID NO:24) produced in Example 5; and [[(B)]] FIG. 6B encoding nucleotide sequence (SEQ ID NO:29).

Please replace the paragraph at page 8, lines 19-21, with the following replacement paragraph:

FIG. 7 (comprising FIGS. 7A and 7B): [[(A)]]FIG. 7A Amino acid sequence of PMC21 NhhA deletion mutant polypeptide (SEQ ID NO:25) produced by splice overlap PCR in Example 6; and [[(B)]] FIG. 7B encoding nucleotide sequence (SEQ ID NO:30).

Please replace the paragraph at page 8, lines 22-24, with the following replacement paragraph:

FIG. 8 (comprising FIGS. 8A and 8B): [[(A)]]FIG. 8A Amino acid sequence of PMC21 NhhA deletion mutant polypeptide (SEQ ID NO:26) produced by splice overlap PCR in Example 7; and [[(B)]]FIG. 8B encoding nucleotide sequence (SEQ ID NO:31).

Please replace the paragraph at page 8, lines 25-27, with the following replacement paragraph:

FIG. 9 (comprising FIGS. 9A and 9B): [[(A)]]FIG. 9A Amino acid sequence of PMC21 NhhA deletion mutant polypeptide (SEQ ID NO: 27) produced by splice overlap PCR in Example 8; and [[(B)]]FIG. 9B encoding nucleotide sequence (SEQ ID NO:32).

Please replace the paragraph at page 10, lines 1-19, with the following replacement paragraph:

FIG. 13 (comprising FIGS. 13A - 13C): Western Immunoblot using anti-NhhA protein mouse sera. In all ~~panels~~ of FIGS. 13A – 13C, lanes 1, 3, 5, 7, contain OMC of Strain over expressing PMC21 NhhA polypeptide, and lanes 2, 4, 6, and 8 contain OMC of strain 2A which does not express NhhA. ~~PanelA~~Figure 13A: Lanes 1 and 2: mouse A inoculated with wild-type PMC21 NhhA at a 1:1000 dilution. Lanes 3 and 4: mouse A inoculated with wild-type PMC21 NhhA at a 1:10.000 dilution. Lanes 5 and 6, mouse B inoculated with wild-type PMC21 NhhA at a 1:1000 dilution. Lanes 7 and 8: mouse B inoculated with wild-type PMC21 NhhA at a 1:10.000 dilution. ~~PanelB~~Figure 13B: Lanes 1 & 2: mouse C inoculated with truncated PMC21 NhhA polypeptide (Example 4) at a 1:1000 dilution. Lanes 3 & 4: mouse C inoculated with truncated PMC21 NhhA polypeptide (Example 4) at a 1:10,000 dilution. Lanes 5 & 6: mouse D inoculated with truncated PMC21 NhhA (Example 4) at a 1:1000 dilution. Lanes 7 and 8: mouse D inoculated with truncated PMC21 NhhA (Example 4) at a 1:1000 dilution. ~~PanelC~~Figure 13C: Lanes 1 & 2: mouse E inoculated with truncated PMC21 NhhA (Example 6) at a 1:1000 dilution. Lanes 3 and 4: mouse E inoculated with truncated PMC21 NhhA (Example 6) at a 1:10,000 dilution. Lanes 5 & 6: mouse F inoculated with truncated PMC21 NhhA (Example 6) at a 1:1000 dilution. Lanes 7 & 8: mouse F inoculated with truncated PMC21 NhhA (Example 6) at a 1:1000 dilution.

Please replace the paragraph at page 10, lines 20-27, with the following replacement paragraph:

FIG. 14 (comprising FIGS. 14A - 14G): Predicted mature NhhA polypeptide deletion mutants. [[A]]FIG. 14A: predicted mature protein described in Example 2 (SEQ ID NO:33); [[B]]FIG. 14B: predicted mature protein described in Example 3 (SEQ ID NO:34); [[C]] FIG. 14C: predicted mature protein described in Example 4 (SEQ ID NO:35); [[D]] FIG. 14D: predicted mature protein described in Example 5 (SEQ ID NO:36); [[E]] FIG. 14E: predicted mature protein described in Example 6 (SEQ ID NO:37); [[F]] FIG. 14F: predicted mature protein described in Example 7 (SEQ ID NO:38); and [[G]] FIG. 14G: predicted mature protein described in Example 8 (SEQ ID NO:39).

Please replace the paragraph bridging pages 42 and 43, which was previously amended in an Amendment filed October 25, 2002, with the following replacement paragraph:

The resulting plasmid, pIP52(PMC21), was linearized by restriction digestion and used to transform *N. meningitidis* strain 7G2 using the method described by Janik *et al*, 1976, Journal of Clinical Microbiology **4** 71. Transformants were selected by overnight incubation at 37 °C in 5% CO_2 on solid media containing 100 µg/ml kanamycin. Kanamycin resistant colonies were selected, subcultured overnight and screened for over-expression of NhhA polypeptide by separating total cell proteins electrophoretically on 10% SDS-PAGE followed by transfer to nitrocellulose membrane using a Semi-Dry Blotter (BioRad). The membrane was then incubated sequentially with rabbit anti-NhhA sera (as described in International Publication WO99/31132) and alkaline-phosphatase conjugated anti-Rabbit IgG (Sigma) before colorimetric detection with NBT/BCIP (Sigma). One clone was isolated which expressed NhhA polypeptide at a higher level compared with the parental strain (FIG. 11). Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14A; SEQ ID NO:33).

Please replace the paragraph at page 43, lines 9-19, which was previously amended in an Amendment filed October 25, 2002, with the following replacement paragraph:

The NhhA protein encoded by the *nhhA* gene of *N. meningitidis* strain H41 was over expressed using the same methods as described in Example 2. This created a recombinant nucleic acid expression construct (open reading frame shown in SEQ ID NO:13) which encodes a polypeptide of 591 amino acids as shown in SEQ ID NO:2. In this example the resulting plasmid pIP52(H41) was linearized, and transformed into *N. meningitidis* strain 7G2. Kanamycin resistant colonies were analysed and one was chosen which when examined by Western immunoblot, demonstrated overexpression of NhhA. (FIG. 11). Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National

Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14B; SEQ ID NO:34).

Please replace the paragraph at page 44, lines 12-24, with the following replacement paragraph:

The resulting amplification product included an *Eag*I and *Bgl*II restriction endonuclease sites. pIP52(PMC21) includes a single *Eag*I site 20 bp upstream of the start of the *nhhA* open reading frame (ORF) and a single *Bgl*II site located within the ORF (see Figure 3B). Therefore, pIP52(PMC21) and the amplification product were subjected to restriction endonuclease digestion with *Eag*I and *Bgl*II, ligated and used to transform competent DH5α strain *E. coli* bacteria; this replaces the *Eag*I/*Bgl*II fragment of pIP52(PMC21) with the PCR product. This created a recombinant nucleic acid expression construct (open reading frame shown in FIG. [[5]]5B; SEQ ID NO:28) which encodes a polypeptide of 512 amino acids as shown in FIG. [[5]]5A (SEQ ID NO:23). This amino acid sequence includes amino acids 1-54 and 134-592 of the wild-type sequence, and thereby deletes the majority of the V1 region, all of the V2 and C2 regions, and part of the C3 region of the wild-type PMC21 NhhA polypeptide.

Please replace the paragraph bridging pages 44 and 45, which was previously amended in an Amendment filed October 25, 2002, with the following replacement paragraph:

Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14C; SEQ ID NO:35). To confirm the presence of a cleavable signal sequence and to confirm the identity of the over expressed protein, outer membrane proteins were semi-purified by isolating the fraction that is insoluble in the detergent sarkosyl.

Please replace the paragraph at 46, lines 1-12, with the following replacement paragraph:

The resulting amplification product contains single *Eag*I and *Stu*I restriction endonuclease sites. The expression construct pIP52(H41) contains these restriction sites.

Therefore, pIP52(H41) and the amplification product were subjected to restriction endonuclease digestion with *Eag*I and *Stu*I, ligated and used to transform competent DH5α strain *E. coli* bacteria; this ligation replaces the *EagI*/*StuI* fragment of pIP52(H41) with the PCR product. This created a recombinant nucleic acid expression construct (open reading frame shown in FIG. [[6]]6B and SEQ ID NO:29) which encodes a polypeptide of 513 amino acids as shown in FIG. [[6]]6A and SEQ ID NO:24. This amino acid sequence includes amino acids 1-54 and 134-593 of the wild-type sequence, and thereby deletes the majority of the V1 region, all of the V2 and C2 regions, and part of the C3 region of the wild-type H41 NhhA polypeptide.

Please replace the paragraph at page 46, lines 17-20, which was previously amended in an Amendment filed October 25, 2002, with the following replacement paragraph:

Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14D; SEQ ID NO:36).

Please replace the paragraph bridging pages 47 and 48, with the following replacement paragraph:

The amplification products HOMP5'/SO-C and SO-D/HO3'AN were purified from an agarose gel following separation by electrophoresis, were mixed, and subjected to further amplification using primers HOMP5' and HO3'AN. The resulting amplification product encodes amino acids 1-52 and 337-591 of wild-type NhhA of PMC21. This amplification product was subjected to restriction digestion with *Eag*I and *Nco*I, and cloned into pCO14K, as described in Example 1. This recombinant molecule contains regions C1 and C5, thus deleting regions V1 to 4 and C2 to 4. The nucleotide sequence of the open reading frame is shown in FIG. [[7]]7B and SEQ ID NO:30, and the predicted polypeptide sequence derived from this nucleotide sequence is shown in FIG. [[7]]7A and SEQ ID NO:25.

Please replace the paragraph at page 48, lines 13-16, which was previously amended in

an Amendment filed October 25, 2002, with the following replacement paragraph:

Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14E; SEQ ID NO:37).

Please replace the paragraph at page 49, lines 14-27, which was previously amended in an Amendment filed October 25, 2002, with the following replacement paragraph:

The amplification products HOMP5'/SO-E and SO-F/HO3'AN will be purified from agarose gel following separation by electrophoresis, and will be mixed, and subjected to further amplification using primers HOMP5' and HO3'AN. The resulting product encodes amino acids 1-52 and 211-591 of wild-type NhhA of PMC21. This amplification product will be subjected to restriction digestion with *Eag*I and *Nco*I, and cloned into pCO14K. This recombinant molecule contains regions C1, C4, V4 and C5 thus deleting regions V1-3 and C2-3. The nucleotide sequence of the open reading frame is shown in FIG. [[8]]8B and SEQ ID NO:31, and the predicted polypeptide sequence derived from this nucleotide sequence is shown in FIG. [[8]]8A and SEQ ID NO:26. Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14F; SEQ ID NO:38).

Please replace the paragraph bridging pages 51 and 52, with the following replacement paragraph:

The amplification products HOMP5'/SO-I and SO-J/HO3'AN will be purified from agarose gel following separation by electrophoresis, and will be mixed, and subjected to further amplification using primers HOMP5' and HO3'AN. The resulting product encodes amino acids 1-52, 103-114, 125-188, 211-229, and 237-591 of wild-type NhhA of strain PMC21. The resulting product will be subjected to restriction digestion with *Eag*I and *Nco*I, and cloned into

pCO14K. This recombinant molecule contains regions C1, C2, C3, C4 and C5, thus deleting regions V1, V2, V3, and V4. The nucleotide sequence of the open reading frame is shown in FIG. [[9]]9B and SEQ ID NO:32, and the predicted polypeptide sequence derived from this nucleotide sequence is shown in FIG. [[9]]9A and SEQ ID NO: 27. Analysis of the predicted amino acid sequence using the computer program SIGCLEAVE (part of the eGCG suite of programs ~~hosted at the World Wide Web site of~~ available from the Australian National Genomic Information Service {ANGIS}) indicates that the first 51 amino acids will be cleaved to produce the mature polypeptide (~~FIG. 14~~FIG. 14G; SEQ ID NO:39).